Assessment of Nonalcoholic Fatty Liver Disease by 1H-MRS Hepatic Lipid Profiling; A Preliminary Animal Study at 9.4T

Yunjung Lee[1] and Hyeonjin Kim[1,2]

[1]*Radiology, Seoul National University Hospital, Seoul, Korea,* [2]*Medical Sciences, Seoul National University, Seoul, Korea*

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) encompasses a spectrum of histologic changes in the liver including steatosis, inflammation, necrosis, fibrosis, and its end-stage, cirrhosis. Recently, it is considered one of the most common liver diseases in adults [1], and yet, biopsy remains the current gold-standard for assessing the disease. Therefore, the development of a means of non-invasively diagnosing the disease is a remaining crucial task [2].

Given a relatively small number of metabolites detectable in the liver with in vivo 1H-MRS and the importance of hepatic lipid composition in chronic liver disease [3, 4], 1H-MRS hepatic lipid profiling (MR-HLP) studies have been reported previously [5, 6], by which lipid saturation and polyunsaturation status of the liver may be obtained. However, due either to the limited disease spectrum studied or poor SNR of spectra at clinical field strength, the applicability of in vivo MR-HLP in NAFLD remains unclear. In this study, we address this issue at 9.4T in CCl4-treated rats with different disease severity. We hypothesized that in vivo MR-HLP at high field may depict the altered hepatic lipid compositions in NAFLD.

MATERIALS AND METHODS

Animal Preparation: The animal research protocol was approved by the IACUC. A total of 31 male Sprague-Dawley rats entered the study. Liver diseases were induced in 23 rats by an i.p. injection of CCl4 mixed with vegetable oil three times per week [7] for 2-10 weeks. There were 8 control rats, among which 4 rats received pure vegetable oil at the same frequency.

1H-MRS: All MRS spectra were collected on a 9.4T animal scanner (Bruker, Germany) by using a STEAM sequence (TE/TM/TR = 2.2/3/5000 ms, spectral width = 5000 Hz, 2048 data points, 128 signal averages). For each animal, spectra were acquired from a total of three voxels (4x4x4 mm^3) in order to account for potential liver heterogeneity.

Histopathology: Livers were harvested and stained with hematoxalin and eosin (HE), and Masson's trichrome (MT) by routine methods. Livers were scored for steatosis, necrosis, inflammation and fibrosis. Severity scores ranged from 0 to 4 except for steatosis, for which a 0-5 scale was used.

MRS Data Analysis: The individual peak areas of lipid resonances (Fig.1) were estimated by using AMARES in MRUI [8]. Then, in vivo MR-HLP parameters such as f_{di}, f_{mono} and f_{sat} were estimated as a measure of the fraction of fatty acids that are diunsaturated, monounsaturated, and saturated, respectively [9]. For each rat these MR measures were estimated for each voxel and the mean values over the three voxels were used in the data analysis.

Statistical Analysis: All statistical analyses were performed using SAS (SAS Institute Inc., USA) except for decision tree analysis, for which Answer Tree (SPSS Inc., USA) was used. First, by using the Wilcoxon rank sum test, the control and treated groups were compared with respect to the histopathologic parameters, age, weight, and the MRS measures. Secondly, Pearson partial correlation was examined between the MR-HLP parameters. Thirdly, partial canonical correlation analysis (PCCA) was performed in order to examine correlations between the histopathologic parameters and the MR-HLP parameters, controlling for age and weight. Fourthly, based on the results from the PCCA, hierarchical cluster analysis (HCA) was performed by using the average linkage method to group animals according to the severity of steatosis and fibrosis. Finally, the efficacy of the MR-HLP parameters in differentiating among those animals grouped by the HCA was evaluated by performing decision tree analysis using the classification and regression tree (CART) algorithm.

RESULTS

There was no difference in average scores for all histopathologic examinations between the two control groups with and without oil treatment (p>0.536 for all), and therefore they were grouped together as control (n=8). Representative water-unsuppressed (a), water-suppressed (b) and estimated (d) spectra are shown in Fig.1 for a control rat, along with those MRUI-fitted individual spectral components (c) and the residual spectra (e).

Comparison between Control and Treated Animal Groups: As summarized in Table 1, the mean histopathologic scores of the treated group were all significantly higher than those of the control group. The treated group had significantly lower f_{di} and significantly higher f_{mono} values with respect to the control group. The MRS-measured total hepatic lipid content (THLC) of the treated group was significantly higher than that of the control group as for the steatosis scores.

Correlations between Histopathologic and MR-HLP Parameters: According to the PCCA, there was a strong correlation between the histopathologic parameters and the MR-HLP parameters (r=0.775, p<0.01). Both steatosis and fibrosis are positively correlated with f_{mono} and negatively correlated with f_{di}. There were negative correlations between f_{di} and f_{mono} (r=-0.775, p<0.01), and between f_{di} and f_{sat} (r=-0.464, p<0.05).

Grouping Animals According to the Severity of Steatosis and Fibrosis: Based on the findings from the PCCA, a HCA was performed to group the animals according to the severity of steatosis and fibrosis. As summarized in Table 2, the clustering analysis classified the rats into 4 groups which were characterized as control rats (cluster 1; n=8), rats with mild steatosis (cluster 2; n=5), rats with severe steatosis but with mild fibrosis (cluster 3; n=8), and rats with severe steatosis and fibrosis (cluster 4; n=10).

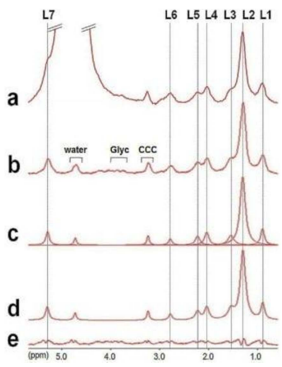

Figure 1. *Representative liver spectra of a normal rat. (a) water-unsuppressed, (b) water-suppressed, (c) fitted spectral components, (d) estimated from those components in (c), (e) residual spectra. (L1-L7: lipid, L1 (methyl), L2 (methylene), L3 (methylene to L5), L4 (allylic), L5 (α-methylene to carboxyl), L6 (diallylic), L7 (methane); CCC: choline-containing-compounds, Glyc: glycogen+glucose)*

Evaluation of the Efficacy of the MR-HLP in Assessing Different Severity of Steatosis and Fibrosis: By performing CART analysis, the efficacy of the MR-HLP in differentiating among those animals grouped by the HCA was evaluated, and the results were summarized in Table 3. In this prediction, the CART model indicated that, first, the separation between the control rats (cluster 1) and the treated rats (clusters 2, 3, 4) was made. Secondly, further separation was made between those rats with severe steatosis and fibrosis (cluster 4) and the rest of the treated rats (clusters 2, 3) including those with severe steatosis and mild fibrosis. The MR-HLP parameters predicted animal groups with prediction accuracy of 62.5% (5/8) for cluster 1, 0% (0/5) for cluster 2, 50% (4/8) for cluster 3, and 100% (10/10) for cluster 4 with overall accuracy of 61.3%.

DISCUSSION

Our results suggest that there are MR-detectable changes in the hepatic lipid composition in vivo in association with the severity of steatosis and fibrosis in diseased livers, which are characterized as decreased f_{di} and increased f_{mono}. Assuming that the three types of fatty acids (diunsaturated, monounsaturated and saturated fatty acids) account for entire fat [6, 9], the decreased f_{di} and the concomitant, increased f_{mono} in diseased liver observed herein are in line with previous in vitro chromatography studies using liver samples from patients with chronic hepatitis C (CHC) or NAFLD [3, 4]. According to our results, it may likely be that f_{di} decreases in association, mainly with increasing f_{mono} and, with increasing f_{sat} to a lesser extent. Given that no significant correlation was observed in our study between the severity of inflammation and the MR-HLP parameters, it may not likely be that MR-HLP helps differentiate between steatohepatitis in its early stages and simple steatosis. Nonetheless, we have shown that the MR-HLP parameters discriminated with 100% prediction accuracy those rats with severe steatosis and fibrosis from the rest of the treated rats including those with severe steatosis and mild fibrosis, which would not have been possible with the simple estimation of THLC alone. In this regard, our study is in support of potential applicability of in vivo MR-HLP at high field in the assessment of NAFLD.

	Control (n=8)	Treated (n=23)	p-value*
Steatosis	0.13 ± 0.35	3.30 ± 1.06	<.0001
Necrosis	0.38 ± 0.52	2.43 ± 0.90	<.0001
Inflammation	0.50 ± 0.53	2.52 ± 0.85	<.0001
Fibrosis	0.00 ± 0.00	2.61 ± 0.78	<.0001
age (week)	13.13 ± 2.42	12.91 ± 2.27	0.9044
weight (g)	482.90 ± 50.91	430.20 ± 39.86	0.0066
THLC	0.04 ± 0.02	0.11 ± 0.06	<.0001
f_{di}	0.45 ± 0.13	0.31 ± 0.11	0.0101
f_{mono}	0.31 ± 0.10	0.42 ± 0.11	0.0074
f_{sat}	0.25 ± 0.06	0.26 ± 0.09	0.8078

Table 1. *Comparison of the control and treated animal groups (*Wilcoxson rank sum test)*

	cluster 1 control (n=8)	cluster 2 mild steatosis (n=5)	cluster 3 severe steatosis and mild fibrosis (n=8)	cluster 4 severe steatosis and fibrosis (n=10)	p-value*
Steatosis	0.13 ± 0.35	1.80 ± 0.45	3.63 ± 0.74	3.80 ± 0.79	<.0001
Necrosis	0.38 ± 0.52	2.40 ± 0.89	1.88 ± 0.64	2.90 ± 0.88	0.0002
Inflammation	0.50 ± 0.53	2.60 ± 0.55	2.13 ± 0.83	2.80 ± 0.92	0.0005
Fibrosis	0.00 ± 0.00	2.80 ± 0.45	1.75 ± 0.46	3.20 ± 0.42	<.0001
f_{di}	0.45 ± 0.13	0.40 ± 0.06	0.27 ± 0.11	0.30 ± 0.11	0.0239
f_{mono}	0.31 ± 0.10	0.35 ± 0.12	0.41 ± 0.09	0.47 ± 0.10	0.0174
f_{sat}	0.25 ± 0.06	0.24 ± 0.10	0.32 ± 0.09	0.23 ± 0.07	0.2232

Table 2. *Classification of animals according to the severity of steatosis and fibrosis (*Kruskal-Wallis test)*

Observed	Predicted				
	cluster 1	cluster 2	cluster 3	cluster 4	total
cluster 1	5	0	1	2	8
cluster 2	1	0	2	2	5
cluster 3	0	0	4	4	8
cluster 4	0	0	0	10	10
total	6	0	7	18	31
accuracy (%)	61.30%				

Table 3. *Prediction of animals with different severity of steatosis and fibrosis by MR-HLP parameters*

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